agronomy

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Boosting Drought Tolerance in Tomatoes through Stimulatory Action of Salicylic Acid Imparted Antioxidant Defense Mechanisms

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Abstract: Drought poses a significant threat to agricultural productivity, particularly affecting economic crops like tomatoes. To address this challenge, various alternatives have been explored, including the use of elicitors or biostimulants such as salicylic acid (SA). This study aims to assess the stimulatory action of SA in alleviating drought stress in tomato plants under greenhouse conditions. The experiment was designed with two main factors: water availability (controlled versus drought) and the foliar application of SA at four different concentrations ranging from 100 to 250 mg L^{-1}. The application of SA, particularly at a concentration of 250 mg L^{-1}, showed promising results in mitigating the adverse effects of drought stress followed by 200 mg L^{-1}. This was evidenced by the increased activity of antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT). Gene expression analysis revealed optimal APX expression with SA application at concentrations of 200 mg L^{-1} or 250 mg L^{-1}. Additionally, the application of SA at 250 mg L^{-1} led to a high accumulation of bioactive compounds without compromising yield. Furthermore, SA application positively influenced both shoot and root weights, with the highest values observed at a concentration of 250 mg L^{-1}. While SA is known to enhance plant tolerance to abiotic stress, further research is needed to fully elucidate its biochemical, physiological, and molecular mechanisms in supporting plant tolerance to drought stress. Utilizing salicylic acid can help growers mitigate environmental stresses, enhancing tomato crop yield and quality. Integrating SA treatments into agriculture offers a sustainable alternative elicitor for ensuring food security under challenging climate conditions.

Keywords: abiotic stress; phytohormone; biostimulants; drought tolerance; gene expression

Citation: Rai, G.K.; Magotra, I.; Khanday, D.M.; Choudhary, S.M.; Bhatt, A.; Gupta, V.; Rai, P.K.; Kumar, P. Boosting Drought Tolerance in Tomatoes through Stimulatory Action of Salicylic Acid Imparted Antioxidant Defense Mechanisms. *Agronomy* **2024**, *14*, 1227. https://doi.org/10.3390/agronomy14061227

Academic Editor: Aisheng Xiong

Received: 6 May 2024
Revised: 18 May 2024
Accepted: 30 May 2024
Published: 6 June 2024

1. Introduction

Climate change poses a significant threat to global food security, driven by rising temperatures and diminishing water resources, which profoundly impact crop productivity and sustainability [1,2]. Among various environmental stressors, drought stress stands out as a major concern, disrupting essential physiological processes and hindering economic yield in crop plants [3]. Tomato (*Solanum lycopersicum* L.), a member of the Solanaceae family and *Lycopersicon* genus, is rich in phytonutrients such as lycopene, vitamin A, vitamin C, and minerals [4,5]. Despite its nutritional value and contributions to human health,

tomatoes are particularly vulnerable to environmental stressors [6,7]. While carotenoids like lycopene offer health benefits, such as cancer prevention and reducing cardiovascular risks, the susceptibility of tomatoes to both abiotic and biotic stresses jeopardizes their quality and yield [8]. Global tomato production exceeds 182.3 million tonnes, with India ranking as the second-largest producer, cultivating over 813 k hectares and yielding nearly 21 million metric tons [8,9]. Andhra Pradesh, Uttar Pradesh, Maharashtra, Karnataka, Bihar, and Orissa are major tomato-producing states in India.

Abiotic stresses such as salinity, UV-B radiation, extreme temperatures, and drought are anticipated to significantly affect the yield of staple food crops by up to 70% [10,11]. Among the abiotic stresses, drought stress is a major constraint in hampering plant productivity by reducing leaf size, limiting stem extension, and curbing root proliferation. It disrupts plant water relations and diminishes water-use efficiency, posing a substantial challenge to plant growth and productivity. One of the primary effects of drought is the reduction in CO_2 assimilation in leaves, mainly caused by stomatal closure, membrane damage, and the disrupted activity of essential enzymes, particularly those involved in CO_2 fixation and adenosine triphosphate (ATP) synthesis. This reduction in CO_2 assimilation is critical, as it directly affects the plant's photosynthetic capacity and energy production [12].

Moreover, drought stress enhances metabolite flux through the photo-respiratory pathway, leading to an increase in the production of reactive oxygen species (ROS). The generation of ROS during photorespiration adds to the oxidative load on plant tissues. These reactive molecules cause significant injury to biological macromolecules, including lipids, proteins, and nucleic acids, further exacerbating the detrimental effects of drought on plant health. The accumulation of ROS and the associated oxidative stress are major deterrents to plant growth under drought conditions. The damage inflicted by ROS impairs cellular structures and functions, ultimately leading to reduced growth and productivity [3].

Drought stress, in particular, imposes severe constraints on tomato cultivation by impeding vegetative growth, seed development, and reproductive processes [2,13]. At the molecular level, drought stress disrupts cellular homeostasis, leading to protein denaturation, membrane damage, and the accumulation of reactive oxygen species (ROS), resulting in oxidative stress-induced cellular damage [14]. Antioxidant defense mechanisms, including enzymes like catalase (CAT), peroxidase (POX), and superoxide dismutase (SOD), play a crucial role in mitigating ROS-mediated damage and maintaining cellular redox balance under drought conditions [15].

To alleviate the adverse effects of drought stress, various management strategies have been explored, including the application of exogenous substances like salicylic acid (SA) [16]. SA, a phenolic compound synthesized endogenously in plants, has garnered attention for its diverse roles in plant growth, development, and stress responses [17]. It acts as a plant hormone and is present in all plants [18]. Studies have shown that the exogenous application of SA in low doses enhances endogenous levels, promoting plant growth and development [19–24]. SA regulates various physiological and metabolic processes, and its application as a biostimulant is emerging as a novel practice to improve crop yield and quality [18]. Moreover, the foliar application of SA enhances growth, photosynthesis, and other physiological and biochemical features in stressed plants [20]. However, plant responses to SA vary depending on cultivar, environmental conditions, and SA concentration [24–26].

Drought stress significantly reduces tomato production globally [27], but there are strategies to enhance drought tolerance in plants. Applying SA to tomatoes has been shown to mitigate the negative impacts of environmental stress [1]. By modulating key metabolic pathways and antioxidant defense systems, SA enhances plant resilience to abiotic stresses, including drought [28]. Considering the above mentioned role, it is hypothesized that SA application can potentially boost the antioxidant defense mechanisms, effectively scavenging reactive oxygen species (ROS) and mitigating oxidative damage to cellular components. This study aims to investigate the potential of SA in alleviating drought stress in tomato plants under high-temperature summer conditions. By optimizing SA

concentrations, we aim to enhance the antioxidant defense machinery and physiological parameters associated with drought tolerance.

2. Materials and Methods

2.1. Planting Material and Experimental Layout

Seeds of the tomato (*Solanum lycopersicum* L.) variety Pusa Ruby were obtained from the National Agri-Food Biotechnology Institute (NABI), Mohali, Punjab, India (30.6674° N, 76.7186° E). The experiment was carried out in pots within a greenhouse setting at the Institute of Biotechnology, formerly known as the School of Biotechnology, SKUAST-Jammu (32.6529° N, 74.8071° E).

Healthy seeds underwent surface sterilization using a 5% sodium hypochlorite solution, followed by thorough washing with distilled water. These seeds were then planted in earthen pots measuring 6 inches in diameter and 7 inches in height, filled with a mixture of sandy loam (organic carbon: 5.2 g kg^{-1}, nitrogen: 0.273 g kg^{-1}, phosphorus: 0.012 g kg^{-1} and potassium: 0.160 g kg^{-1}) and farmyard manure in a 6:1 ratio. The experiment was arranged in a randomized complete block design with four replicates. At the 15-day stage, the seedlings were uprooted and transferred to maintained pots. The environmental conditions included an average temperature of 35 ± 2 °C, a humidity of 80 ± 5%, and a day/night photoperiod of 10 h. Stress was induced by withholding water till temporary wilting occurred, while control plants received regular watering after transplanting to maintain optimum moisture content. At the vegetative stage (25 days after transplanting), the foliar application of SA was administered at various concentrations (100, 150, 200, and 250 mg L^{-1}) prior to water stress, with solutions prepared using distilled water containing 0.02% Tween 20. The treatment-wise foliar application of SA was performed in the early morning (from 9:00 AM to 10:00 AM) to enhance absorption and ensure a lasting effect. A hand-operated sprayer was used to uniformly apply either distilled water for control plants or the salicylic acid solution to each experimental unit. Biochemical and molecular observations were recorded after five days of drought induction, while morphological observations were recorded at the end of the experiment.

2.2. Preparation of Salicylic Acid Solution for Foliar Application

A stock solution containing 750 mg L^{-1} of SA was prepared by dissolving 750 mg of SA powder in 100 mL of 96% ethanol and using distilled water to reach a final volume of 1000 mL. From this stock solution, solutions with concentrations of 100 mg L^{-1}, 150 mg L^{-1}, 200 mg L^{-1}, and 250 mg L^{-1} of SA were obtained, respectively.

2.3. Assessment of Morphological Parameters

The growth was evaluated by measuring parameters such as plant height (in centimeters), leaf area, and the fresh and dry weights of shoots and roots. Plant height was measured using a ruler to determine the elongation of the stems, with measurements taken from at least three separate plants in each treatment group. The average leaf area was determined using a leaf area meter (Bio-Science, CI-202, Mainz, Germany), which measured the combined area of all leaves from two plants per pot. After removing the shoots from the pots, the roots were carefully washed to remove any sand particles. Both the shoot and root tissues were then rinsed with distilled water and dried using tissue paper. Subsequently, their fresh and dry weights were recorded using a digital balance. To obtain the dry weights of roots and shoots, plant materials were dried in an oven (Macro Scientific, MSW-213, Delhi, India) at 60 °C.

2.4. Assessment of Physiological Parameters

2.4.1. Leaf Membrane Stability Index

The leaf membrane stability index (LMSI) was determined according to the method outlined by Premachandra et al. [29]. Leaf discs weighing 200 mg were rinsed with deionized water and then placed into tubes containing 15 mL of double-ionized water,

divided into two sets. The first set was allowed to incubate for 2 h at 25 °C, after which the electrical conductivity of the solution (EC1) was measured. The second set underwent heating in a water bath at 95 °C for 60 min, and the resulting conductivity (EC2) was recorded. The MSI was calculated using the formula given below:

$$\text{MSI} = [1 - (\text{EC1}/\text{EC2})] \times 100 \quad (1)$$

2.4.2. Leaf Relative Water Content

The leaf relative water content (RWC) was assessed gravimetrically using the procedure described by Galmes et al. [30]. Initially, the fresh weight of the leaves was measured. Subsequently, the leaves were floated in distilled water in Petri dishes at 4 °C for 24 h, and their weight was recorded again to determine the turgid weight. The dry weight of the leaves was determined by drying them for 48 h at 90 °C. The RWC (%) was calculated using the formula:

$$\text{RWC\%} = (\text{FW} - \text{DW})/(\text{TW} - \text{DW}) \times 100 \quad (2)$$

where FW represents fresh weight, DW represents dry weight, and TW represents turgid weight.

2.5. Assessment of Biochemical Parameters

2.5.1. Lipid Peroxidation

The lipid peroxidation levels were determined by assessing malondialdehyde equivalents following the method outlined by Hodges et al. [31]. In this process, 0.5 g of leaf tissue was homogenized in 80% ethanol using a mortar. The resulting homogenate underwent centrifugation at 3000× *g* for 10 min at 48 °C. The pellet obtained was then extracted twice using the same solvent. The supernatants were combined, and 1 mL of this solution was mixed with an equal volume of a solution containing 20% trichloroacetic acid, 0.01% butylated hydroxytoluene, and 0.65% thiobarbituric acid in a test tube. The mixture was heated to 95 °C for 25 min and subsequently cooled to room temperature. Absorbance readings of the samples were taken at wavelengths of 440 nm, 532 nm, and 600 nm. The concentration of malondialdehyde (MDA) was determined using an extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as nmol of MDA per gram of fresh weight.

2.5.2. Antioxidant Enzyme Activity

A total of 300 mg of fresh leaf mass was ground in liquid nitrogen and then dissolved in 100 mM sodium phosphate buffer (pH 7.4) containing 1% PVP (polyvinyl pyrrolidone) and 0.5% (*v*/*v*) Triton-X 100. The resulting homogenate was centrifuged at 20,000 rpm for 20 min at 4 °C. The supernatant was collected and stored at −20 °C for protein determination using NanoDrop™ 8000 (Thermo Fisher Scientific, Waltham, MA, USA, sourced; Delhi, India), while the specific activities of antioxidant enzymes including SOD, APX, and CAT were extracted and assessed following the methods outlined by Jogeswar et al. [32].

Superoxide Dismutase (SOD) activity was determined according to the method described by Beauchamp and Fridovich. In this assay, 0.1 mL of enzyme extract was added to a reaction mixture containing 1.5 mL of 50 mM sodium phosphate (pH 7.8), 0.3 mL of 130 µM methionine, 0.3 mL of 750 µM nitro-blue tetrazolium (NBT), 0.3 mL of 100 µM EDTA-Na2, 0.300 mL of 20 µM riboflavin, and 100 µL of distilled water. After exposure to light at 4000 flux for 20 min, the absorbance of the sample was measured at 560 nm. SOD activity was expressed as the amount of enzyme required for a 50% inhibition of NBT reduction.

Ascorbate peroxidase (APX) activity was determined by homogenizing one gram of leaf samples in liquid nitrogen with sodium phosphate buffer (pH 7.2) and polyvinylpolypyrrolidone using a pre-chilled mortar and pestle. The crushed sample was then centrifuged for 20 min at 6700 rpm, and the resulting supernatant was used as a crude enzyme extract. The reaction mixture was prepared by combining 1500 µL phosphate buffer, 20 µL EDTA, 1000 µL sodium ascorbate, and 20 µL enzyme extract. The reaction was initiated by adding

480 µL hydrogen peroxide, and the decrease in optical density was measured at 290 nm against a blank every two minutes.

Catalase (CAT) activity was assessed following the procedure outlined by Chance and Machly (1955) [33]. A redox technique was employed to evaluate CAT activity. Specifically, 200 µL of enzyme extract was added to a reaction mixture consisting of 1.5 mL of 50 mM sodium phosphate (pH 7.8), 300 µL of 0.1 M H_2O_2, and 1.0 mL of distilled water. The reduction in H_2O_2 was monitored, and the change in absorbance at 240 nm per minute indicated CAT activity.

2.6. Gene Expression Analysis

Plant leaf samples were collected in Trizol, and the total RNA was isolated following the manufacturer's instructions using the Gene JET Plant RNA Purification Kit (Thermo Fisher Scientific, USA, sourced; Mumbai, India). Subsequently, cDNA was synthesized using the Maxima H minus first-strand cDNA synthesis kit with dsDNase (Thermo Fisher Scientific, USA). Sequences for SOD, APX, CAT, and Actin (used as a housekeeping gene) were retrieved from the Sol genome browser and NCBI GeneBank, and alignment was conducted using MAFFT alignment software (v6.240). Conserved sequences were identified, and primers were designed using primer3 software (v 4.1.0). An expression analysis was conducted with four biological and four technical replicates. Quantitative PCR (qPCR) was performed in 96-well plates (CFX 96 Touch™ Real-Time PCR, Bio-Rad, Hercules, CA, USA). Each reaction had a final volume of 20 µL, consisting of 10 µL of 2X SYBR Green Master Mix (Thermo Scientific, Waltham, MA, USA), 6 µL of cDNA samples, and 200 nM primers. The cycling conditions were set as follows: initial denaturation at 95 °C for 3 min, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 1 min. The housekeeping gene Actin was used as an internal reference to normalize expression levels [34]. The specificity of amplification was confirmed through melting curve analysis. Relative expression levels were determined using the $2^{\Delta\Delta CT}$ method [35].

2.7. Statistical Analysis

All recorded parameters were analysed and the impact of the treatments was assessed using one-way analysis of variance (ANOVA). Mean comparisons were conducted using Duncan's multiple range test. Statistical significance was determined at a threshold of $p \geq 0.05$ using SPSS 16.0, also all graphs were plotted using Ms-Excel 2016 (v2404).

3. Result

3.1. Morphological Parameters

3.1.1. Plant Height

The mean data, along with standard deviation, demonstrate the impact of various doses of SA on plant height (Table 1). The interactive effect of these variables was found to be significant ($p \geq 0.05$). Among the different application methods, the tallest plants (79.72 ± 1.03 cm) were observed in the control group (T_0), while the shortest height (52.40 ± 0.47 cm) was recorded for plants subjected to drought without SA treatment (0 mg L^{-1}). Considering the interaction between the modes of SA application and concentrations, the tallest heights (72.11 ± 0.46 cm) were noted in plants treated with 250 mg L^{-1} of SA alongside drought, whereas the shortest height (53.90 ± 0.47 cm) was observed in plants treated with 100 mg L^{-1} of SA alongside drought.

Table 1. List of antioxidant gene primers for gene expression profiling.

Primer Name	Sequence	Annealing Temp. (°C)
β-actin (F)	TTGACTGAGGCACCACTTAACCCT	68.7
β-actin (R)	GCTTTCAGGTGGTGCAACGACTTT	71.0
SOD (F)	CACGTCTTCAAAGCAAGTGG	63.5
SOD (R)	CTAAGAAGAAGGGCATTCTTTGGCAT	68.7
CAT (F)	GATGAGCACACTTTGGAGCA	64.1
CAT (R)	TGCC CTTCTATTGTGGTTCC	63.8
APX (F)	GAAACTCAGAGGACTCATTGCTGAGAAGAATTG	72.9
APX (R)	GAAACTGCTCCCTAATGGGCTCCAAGAG	73.9

SOD: Superoxide dismutase; CAT: Catalase; APX: Ascorbate peroxidase; F: Forward; R: Reverse.

3.1.2. Leaf Area

The mean data, along with the standard deviation, illustrate the impact of different doses of SA application on leaf area (Table 1). Notably, the interactive effect of these variables was found to be significant. Highest leaf area (1651.93 ± 0.17 cm^2) was observed in plants under control conditions, while the lowest leaf area (1340.92 ± 0.35 cm^2) was recorded for plants subjected to drought stress without SA treatment (0 mg L^{-1}). Regarding SA concentrations, the highest leaf area (1649.97 ± 0.63 cm^2) was noted in plants treated with 250 mg L^{-1} of SA alongside drought, whereas the lowest leaf area (1356.05 ± 0.42 cm^2) was observed in plants treated with 100 mg L^{-1} of SA under drought conditions. These results highlight the diverse effects of SA application on leaf area, with implications for plant response to drought stress.

3.1.3. Shoot Fresh and Dry Weight

Substantial differences were observed among control, drought, and various SA application levels before drought treatments in terms of tomato shoot fresh and dry weight (Table 1). The dose of SA application significantly influenced the shoot fresh and dry weight outcomes. The highest shoot fresh weight (26.85 ± 0.012 g) was observed in the 200 mg L^{-1} SA treatment combined with drought (T_4), while the lowest (18.52 ± 0.012 g) was recorded in drought-only conditions without SA application. Concerning shoot dry weight, the highest (13.11 ± 0.0 g) was observed in plants treated with 250 mg L^{-1} SA alongside drought, followed by (9.63 ± 0.01 g) in those treated with 100 mg L^{-1} SA alongside drought. Conversely, the lowest shoot dry weight (8.37 ± 0.98 g) was noted in drought conditions without SA (0 mg L^{-1}). Compared to control plants, both the shoot fresh and dry weights were significantly reduced under drought conditions. Notably, increasing SA doses (100–250 mg L^{-1}) exhibited an upward trend in tomato shoot fresh and dry weights.

3.1.4. Root Fresh and Dry Weight

The mean data analysis reveals the significant effects of different levels of SA on root fresh and dry weight (Table 2). The greatest root fresh weight (27.57 ± 0.38 g) was noted for the 250 mg L^{-1} treatment (T_5), whereas the lowest root fresh weight (17.42 ± 0.09 g) was recorded for the control group. Similarly, concerning root dry weight, the application of 250 mg L^{-1} SA alongside drought resulted in the highest root dry weight (3.36 ± 0.01 g), while the control plants exhibited the lowest root dry weight (1.96 ± 0.01 g). Notably, there was a clear increasing trend in root dry weight observed with increasing SA application levels, surpassing that of the control treatment without SA.

Table 2. Dynamics of tomato growth under drought stress and SA treatments.

Treatments	Plant Height (cm)	Leaf Area (cm^2)	Stem Fresh Weight (g)	Stem Dry Weight (g)	Root Fresh Weight (g)	Root Dry Weight (g)
T_0-Control	79.72 ± 1.03 ^{a}	1651.93 ± 0.17 ^{a}	26.70 ± 0.473 ^{a}	13.1 ± 0.14 ^{a}	17.42 ± 0.09 ^{e}	1.96 ± 0.01 ^{f}
T_1-Drought	52.40 ± 0.47 ^{e}	1340.92 ± 0.35 ^{f}	18.52 ± 0.012 ^{d}	8.37 ± 0.98 ^{ae}	19.99 ± 0.06	2.10 ± 0.08 ^{e}
T_2-100 mg L^{-1} + Drought	53.90 ± 0.47 ^{d}	1356.05 ± 0.42 ^{e}	19.66 ± 0.018 ^{c}	9.63 ± 0.01 ^{d}	24.42 ± 0.09 ^{c}	2.63 ± 0.00 ^{d}
T_3-150 mg L^{-1} + Drought	64.9 ± 0.38 ^{c}	1562.97 ± 0.69 ^{d}	24.54 ± 0.012 ^{b}	12.55 ± 0.0 ^{c}	26.46 ± 0.00	3.11 ± 0.00 ^{c}
T_4-200 mg L^{-1} + Drought	72.0 ± 0.45 ^{b}	1640.02 ± 0.47 ^{c}	26.85 ± 0.012 ^{a}	12.84 ± 0.0 ^{b}	27.35 ± 0.12 ^{a}	3.22 ± 0.01 ^{b}
T_5-250 mg L^{-1} + Drought	72.11 ± 0.46 ^{b}	1649.97 ± 0.63 ^{b}	26.77 ± 0.018 ^{a}	13.11 ± 0.0 ^{a}	27.57 ± 0.38 ^{a}	3.36 ± 0.01 ^{a}

The values are the mean of four replicates with ±standard deviation followed by different letters for each parameter, which differ significantly as per DMRT ($p < 0.05$).

3.2. Physiological Parameters

3.2.1. Leaf Membrane Stability Index (LMSI%)

Different doses of SA had a significant impact on the LMSI content of tomatoes, as indicated in Table 2. Among the various application doses, plants treated with 200 mg L^{-1} exhibited the highest LMSI content, while those subjected to drought showed the least impact (40.05 ± 0.10%). An analysis of the data clearly revealed that SA, at a concentration of 200 mg L^{-1}, resulted in the highest LMSI content (48.25 ± 0.01%), followed closely by 150 mg L^{-1} (48.14 ± 0.01%). The treatments with SA at the levels of 150 mg L^{-1} and 200 mg L^{-1} showed statistically similar effects. Conversely, the application of exogenous SA significantly enhanced LMSI content compared to stressed plants, as shown in Table 3.

Table 3. Dynamics of physiochemical parameters of tomatoes under drought stress and SA treatments.

Treatments	LMSI (%)	LRWC (%)	Lipid Peroxidation (nmol g^{-1} fw)
T_0-Control	46.92 ± 0.09 ^{d}	73.92 ± 0.09 ^{a}	0.67 ± 0.0 ^{f}
T_1-Drought	40.05 ± 0.10 ^{f}	58.10 ± 0.08 ^{f}	2.36 ± 0.0 ^{a}
T_2-100 mg L^{-1} + Drought	43.52 ± 0.01 ^{e}	62.10 ± 0.08 ^{e}	1.97 ± 0.0 ^{b}
T_3-150 mg L^{-1} + Drought	48.14 ± 0.01 ^{b}	66.92 ± 0.09 ^{d}	1.12 ± 0.0 ^{c}
T_4-200 mg L^{-1} + Drought	48.25 ± 0.01 ^{a}	70.14 ± 0.00 ^{c}	0.98 ± 0.0 ^{e}
T_5-250 mg L^{-1} + Drought	47.13 ± 0.01 ^{c}	71.58 ± 0.00 ^{b}	1.10 ± 0.0 ^{d}

The values are the mean of four replicates with ±standard deviation followed by different letters for each parameter, which differ significantly as per DMRT ($p < 0.05$). LMSI: Leaf membrane stability index; LRWC: Leaf relative water content.

3.2.2. Leaf Relative Water Content (%)

The LRWC data presented in Table 2 reflect significant variations, influenced by both the doses of application and the levels of SA. It is evident from the average values that the highest LRWC (73.92 ± 0.09) was observed in the control group with no foliar treatment, while the lowest LRWC (58.10 ± 0.08) was recorded in plants subjected to drought stress without foliar treatment. Concerning SA concentrations, the maximum LRWC (71.58 ± 0.00) was observed in plants treated with 250 mg L^{-1} + Drought, followed closely by the LRWC (70.14 ± 0.00) in plants treated with 200 mg L^{-1} + Drought. The LRWC exhibited a dose-dependent increase with rising SA treatment levels. Conversely, the application of exogenous SA resulted in an improved LRWC compared to drought-stressed plants, albeit still lower than that of the control plants.

3.2.3. Lipid Peroxidation (MDA nmol g^{-1} fw)

The different application methods and concentrations of SA significantly influenced the lipid peroxidation levels in tomatoes, as demonstrated in Table 2. An analysis of the data reveals that the concentration of SA at 100 mg L^{-1} + Drought exhibited the highest lipid peroxidation content (1.97 ± 0.0), followed by 150 mg L^{-1} (1.12 ± 0.0). In contrast, drought-stressed plants exhibited higher levels of malondialdehyde (MDA) (2.36 nmol g^{-1} fw)

compared to the control (0.67 nmol g^{-1} fw). Notably, treatment with SA at 200 mg L^{-1} resulted in a significant reduction in MDA content (Table 2). Administering SA prior to drought stress demonstrated a more pronounced effect on lipid peroxidation.

3.2.4. Anti-Oxidant Enzyme Activity

The activity of SOD enzyme in tomatoes displayed a dose-dependent increase with rising SA treatment levels. Control plants exhibited an SOD activity ranging from 24.10 to 31.23 units min^{-1} g^{-1} FW, with the highest activity recorded in plants treated with 250 mg L^{-1} SA. This effect was even more pronounced when SA-treated plants underwent drought stress, resulting in the highest SOD activity observed in drought-stressed plants treated with higher SA levels (Table 3). Administering SA in conjunction with drought stress led to a greater increase in SOD activity.

APX activity ranged from 117.94 to 172.28 µmol min^{-1} mg^{-1} FW, with the highest activity recorded in plants treated with the highest SA concentration (250 mg L^{-1}). Conversely, the lowest activity was observed in untreated control plants grown under normal conditions. Increasing levels of SA treatment resulted in an upward trend in APX activity among tomato plants (Table 4).

Table 4. Salicylic Acid and Antioxidant Enzymes in Drought-Stressed Tomatoes.

Treatments	Superoxide Dismutase (Unit min^{-1} g^{-1} FW)	Ascorbate Peroxidase (µmol min^{-1} mg^{-1} FW)	Catalase (µmol min^{-1} mg^{-1} FW)
Control	24.10 ± 0.08 ^{e}	117.94 ± 0.87 ^{f}	31.92 ± 0.43 ^{f}
Drought	27.13 ± 0.12 ^{d}	120.59 ± 0.91 ^{e}	34.57 ± 0.54 ^{e}
Drought + 100 mg L^{-1}	28.29 ± 0.12 ^{c}	134.90 ± 0.95 ^{d}	49.52 ± 0.71 ^{d}
Drought + 150 mg L^{-1}	30.08 ± 0.11 ^{b}	148.7 ± 0.68 ^{c}	62.69 ± 0.50 ^{c}
Drought + 200 mg L^{-1}	30.95 ± 0.50 ^{a}	152.86 ± 0.22 ^{b}	75.39 ± 0.47 ^{b}
Drought + 250 mg L^{-1}	31.23 ± 0.01 ^{a}	172.28 ± 0.53 ^{a}	89.16 ± 0.79 ^{a}

The values are the mean of four replicates with ±standard deviation followed by different letters for each parameter differ significantly as per DMRT ($p < 0.05$).

Regarding catalase (CAT) enzyme activity in tomatoes, significant variations were observed ($p \geq 0.05$) across different treatments (Table 3). Control plants exhibited lower CAT activity compared to plants subjected to drought stress and SA treatments. The application of increasing levels of SA resulted in increased CAT enzyme activity. The highest catalase enzyme activity (89.16 µmol min^{-1} mg^{-1} FW) was noted with the application of 250 mg L^{-1} SA, followed by 200 mg L^{-1} (75.39 µmol min^{-1} mg^{-1} FW) and 150 mg L^{-1} (62.69 µmol min^{-1} mg^{-1} FW) before drought stress, while the lowest activity (31.92 µmol min^{-1} mg^{-1} FW) was recorded under control conditions.

3.3. Anti-Oxidant Genes Expression Analysis

Under drought stress, the gene expression of SOD was significantly elevated compared to the control conditions, while SA pre-treated plants exhibited lower expression levels than those under drought stress alone. Furthermore, increasing levels of SA application corresponded to decreasing trends in SOD gene expression, with treatments at 200 mg L^{-1} and 250 mg L^{-1} showing comparable effects. RT-PCR analysis was standardized using Actin as a housekeeping gene alongside internal control samples. The highest gene expression of APX was observed under stressed conditions compared to the control. SA played a role in regulating gene expression, with expression levels reduced under drought stress. The pre-application of SA before drought stress decreased the relative fold expression of the APX gene up to 200 mg L^{-1}, beyond which further increases in SA levels (250 cmg L^{-1}) did not alter the expression significantly. The SA level of 200 mg L^{-1} appeared to be optimal for relative fold expression. Actin was utilized as the housekeeping gene for transcript profiling in this study. CAT gene expressions were higher under drought stress compared to control conditions. Following SA treatment before drought stress, a decreasing trend

in CAT gene expression was observed up to 250 mg L^{-1}, with the optimum relative fold expression noted at 200 mg L^{-1}. Actin served as the housekeeping gene for standardization in this study and the results are depicted in Figure 1.

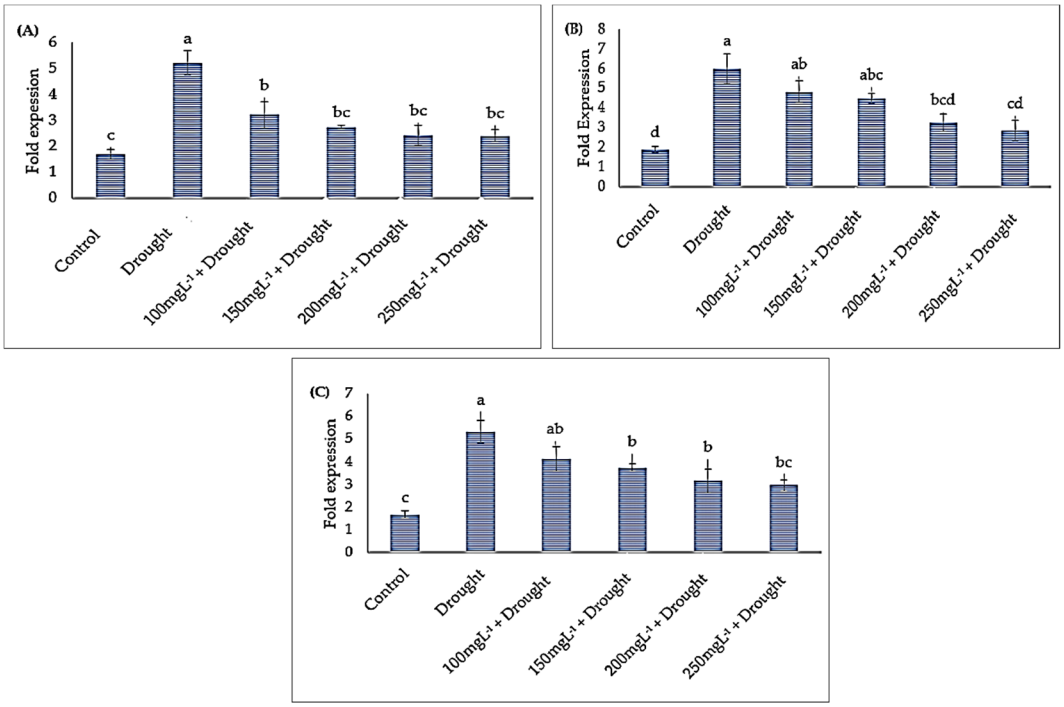

Figure 1. Salicylic acid-mediated antioxidant gene expression profiling in tomatoes under drought stress using qRT PCR. Antioxidant genes viz., (**A**) Ascorbate peroxidase (**B**) Superoxide dismutase (**C**) Catalase. All the data are presented as mean ± standard error of replicates (three biological and four technical replications) and different letters above each bar indicate a significant difference between treatments ($p < 0.05$, one way ANOVA).

4. Discussion

Tomatoes (*Solanum lycopersicum* L.) are highly valued for their rich content of health-beneficial bioactive compounds, such as phenolics, carotenoids, and vitamins, making them essential in dietary supplements. However, the potential yield and quality of tomatoes are significantly hindered by various abiotic stresses, with water scarcity being a predominant challenge. Water deficit poses a severe threat, exacerbating oxidative stress within plants by disrupting the balance in reactive oxygen species (ROS) production, including singlet oxygen ($^1O_2$), superoxide anion radicals (O^{2-}), hydroxyl radicals (OH^-), and hydrogen peroxide (H_2O_2). The excessive accumulation of ROS damages crucial cellular components such as DNA, RNA, proteins, and lipids, thereby impairing normal cellular functions. To cope with this oxidative stress, plants produce signaling molecules like SA, which acts as a key player in enhancing water-use efficiency and adaptation to drought stress [36].

Exogenous application of SA has been shown to enhance plant growth and development in earlier studies [28,37]. In our research, the foliar application of SA particularly at 250 mg L^{-1} or 200 mg L^{-1} increased plant height by over 37% compared to drought-stressed tomato plants without SA treatment. Additionally, applying SA at 250 mg L^{-1} significantly boosted both plant and root biomass compared to the control. These findings are consistent with those of Iosob et al. [38], who investigated the effects of SA on the growth and yield of tomatoes.

The application of exogenous SA has demonstrated to enhance antioxidant effectiveness across diverse biological contexts. SA is pivotal in the regulation of reactive oxygen species (ROS), such as hydrogen peroxide. Furthermore, SA induction has been shown to elevate the regulation of antioxidant enzymes in response to oxidative stress [39]. The

application of exogenous SA has been found to bolster the antioxidant defense system under stress conditions, with higher concentrations, up to 250 mg L^{-1}, showing potential in enhancing the resistance of tomato plants to water stress [13]. Jahan et al. [40] also reported that the exogenous application of SA improved the antioxidant defense mechanisms in tomatoes under heat stress. Drought stress may lead to an elevation in lipid peroxidation (MDA) and electrolyte leakage (EL) levels, potentially due to cellular dehydration [41], thus inducing oxidative stress on cell membranes and it serves as a potential marker of oxidative stress and is crucial for evaluating drought tolerance in crops.

Membrane stability was noted to significantly decrease under drought stress; however, SA-treated tomato plants enhanced the membrane stability index (MSI) by reducing membrane damage due to a reduction in lipid peroxidation. In this study, foliar applications of SA played pivotal roles in influencing the leaf membrane stability index (LMSI), the leaf relative water content (LRWC), and lipid peroxidation levels. Particularly, SA treatments, notably at a concentration of 200 mg L^{-1}, exhibited enhanced LMSI content, indicating improved membrane stability under drought stress. Similar findings were reported by Hayat et al. [42] and Aires et al. [43], who evaluated the foliar application of salicylic acid (SA) to improve the membrane stability index (MSI) and leaf water retention capacity (LWRC), while reducing the lipid peroxidation caused by oxidative stress in tomatoes. Additionally, the application of SA resulted in dose-dependent increases in LRWC, though these levels remained below those of the control plants, indicating a partial alleviation of water deficit stress. Moreover, SA treatments significantly reduced lipid peroxidation levels, especially at 200 mg L^{-1} concentration, highlighting SA's potential in mitigating oxidative stress induced by drought. Furthermore, the activity of antioxidant enzymes such as Superoxide Dismutase (SOD), Ascorbate Peroxidase (APX), and Catalase (CAT) increased significantly in SA pre-treated plants during drought stress [44]. These findings align with previous research, suggesting that SA application enhances antioxidant enzyme activity in tomato plants [1]. Moreover, there is a connection between CAT and lipid peroxidation (MDA) in tomatoes, consistent with earlier studies [1,45]. SA exerts its stress-mitigating effects by stimulating the expression of antioxidant genes and enhancing the activity of antioxidant enzymes [46]. This study indicates that the reduced electrolyte leakage (EL) and improved membrane stability in SA-treated plants may protect cells from oxidative damage through the regulation of SOD, APX, and CAT, thereby enhancing the scavenging machinery within cells. Drought stress typically inhibits tomato plant growth due to ROS overproduction and osmotic imbalance, leading to reduced vigor. However, SA-treated plants displayed a lower expression of antioxidant genes under drought stress conditions, indicating improved membrane integrity, water status, and physiological functions. This protective mechanism minimizes the negative effects of oxidative stress during drought conditions. Furthermore, the upregulation of ROS scavenging and osmotic regulating genes further supports the role of SA in enhancing plant resilience to drought stress [47,48].

In this study, the elevated expression of the superoxide dismutase (SOD) gene under drought stress indicated the plant's response to oxidative stress, which was mitigated by SA pre-treatment, particularly at concentrations of 200 mg L^{-1} and 250 mg L^{-1}. Similarly, the expression of the ascorbate peroxidase (APX) and catalase (CAT) genes was upregulated under drought stress, demonstrating their roles in scavenging reactive oxygen species. SA treatment modulated gene expression, with a notable reduction observed at optimal concentrations, suggesting the regulatory effect of SA on antioxidant pathways [49]. These findings underscore the potential of SA in regulating antioxidant gene expression to enhance plant stress tolerance, offering valuable insights for developing strategies to improve crop resilience in water-limited environments.

5. Conclusions

The foliar application of SA effectively enhances the antioxidant system, bolstering crucial enzymes like superoxide dismutase, ascorbate peroxidase, and catalase, particularly under stressful conditions. The research findings highlight the potential of SA not only in

alleviating drought-induced stress but also in improving overall plant vigor and resilience. In this study, optimized concentrations of SA led to significant improvements in both the growth and quality of tomatoes, especially during challenging summer months. Among the concentrations tested, foliar treatments with 250 mg L^{-1} SA showed the most promising results, enhancing various morpho-physiological parameters crucial for plant development as well as significantly increasing the activity of antioxidant enzymes and the expression of antioxidant genes. These findings have significant implications for agricultural practices, offering a practical strategy for farmers to enhance tomato production, particularly in regions prone to drought stress during the summer season. By harnessing the beneficial effects of salicylic acid, growers can potentially mitigate the adverse impacts of environmental stresses, thus enhancing both the yield and quality of tomato crops. Incorporating SA treatments into agricultural protocols presents a promising avenue for sustainable crop management and ensuring food security in challenging climatic conditions.

Author Contributions: Conceptualization, G.K.R., P.K.R. and P.K.; methodology, I.M. and S.M.C.; software, V.G., A.B. and D.M.K.; validation, G.K.R., I.M. and D.M.K.; formal analysis, I.M., S.M.C., G.K.R., P.K.R. and P.K.; investigation, G.K.R. and I.M.; resources, I.M. and S.M.C.; data curation, I.M.; writing—original draft preparation, G.K.R., I.M. and D.M.K.; and editing, G.K.R., P.K.R., V.G., A.B. and P.K.; visualization, I.M. and S.M.C.; supervision, G.K.R. and P.K.R.; acquisition, G.K.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Conflicts of Interest: The authors declare no conflicts of interest.

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